The Egg Mass and Veligers of *Limacina helicina* Phipps

BY

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(2 Text figures)

INTRODUCTION

In the summer of 1966, while at the Friday Harbor Laboratories, Friday Harbor, Washington, I had an opportunity to observe the spawn and the veligers of an euthecosomatous pteropod, *Limacina helicina* (Phipps, 1774), in the laboratory. In view of the widespread occurrence of this genus throughout the world oceans, and in particular of this species in the polar and subpolar seas, it is rather surprising that there should be so little information on the breeding of these animals. More than 100 years ago, Van Beneden (1839) described the structure of the reproductive system of *L. arctica (L. helicina)* and Pelseneer (1888) gave a detailed account of the morphology and anatomy of this species. Massy (1920) observed many spawning specimens in the material collected in the Antarctic Ocean. She described the spawn-mass as a transparent, glutinous material connecting many white, oblong bodies, each of which averaged 132μ by 88μ. Besides this brief note, there are apparently no other observations on the spawn and veligers of this species. Thus, the present observations are of some interest.

McGowan (1963), in an extensive study of the distribution of *Limacina helicina* in the subarctic North Pacific, recognized two morphologically dissimilar, well-defined varieties: Variety A restricted to the Northwest Pacific, and Variety B limited to the Northeast Pacific. Where the boundaries of the distributions of these two varieties meet, intergrades AB were found. The present observations were made on Variety B.

MATERIALS and METHODS

The animals were collected with a 1.5 m diameter ring-net, in Saanich Inlet, British Columbia, Canada. The net was towed vertically from 100 m to the surface at night and the contents of the net were poured and diluted in several polyethylene trays. The animals were sorted out immediately and were placed in 32-oz glass jars with screw caps, which were immersed in tanks with running sea water. In the laboratory, the animals were maintained in similar containers with unfiltered sea water to which a mixture of *Platymonas* sp. and *Skeletonema costatum* (Greville) Cleve, 1878 was added. The water was changed once every two days. The temperature of the water was about 13°C (±2°C).

After two days in the laboratory, the majority of the animals spawned, perhaps in response to the higher temperature of the laboratory sea water. Copulation and the actual act of spawning were not observed. Most animals released egg ribbons which were found floating in the containers. In few animals, the egg ribbons were still attached to the body and the animals appeared to be having considerable difficulty in swimming. Instead of smooth, swift strokes of the wings, very awkward flapping movement was observed. In such cases, the egg ribbons were carefully removed from the animals. Few animals spawned again after about 20 days. The egg ribbons were observed under the microscope and their linear dimensions and the total number of eggs were estimated. The egg ribbons were then transferred to 6-oz finger bowls containing glass-fiber-filtered sea water. Thirty mg of streptomycin sulfate per liter was added to the water to control the bacterial growth. The finger bowls were placed on the water table with running sea water.

After the eggs had hatched, the veliger larvae were divided into two groups. One group was given unfiltered sea water, while the other group was maintained in the filtered sea water, supplemented with *Platymonas* sp. Periodically, a few larvae were removed from each group and were anesthetized partially with 1% chloretone or 1% magnesium sulphate, on a depression slide. Drawings

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were made with the aid of either a Whipple disc or a camera lucida.

OBSERVATIONS

Adults:

Most animals were healthy for about 22 to 28 days in the laboratory. The animals actively swam up to the surface of the water in a broad spiral path and passively sank to the bottom, by a method very similar to that of *Limacina retroversa* Fleming, 1823 (Morton, 1954). Healthy specimens were almost transparent except for the deep-brown gonads. Unhealthy specimens soon acquired a dark-brown color around the edges of the wings and the visceral mass, became inactive, and died.

The genus *Limacina* is known to be a filter-feeder (Morton, 1954). The gut contents of the specimens preserved in the field showed a few remains of diatoms, *Skeletonema costatum, Chaetoceros* spp., and *Thalassiosira* spp.; but dinoflagellates *Prorocentrum* sp., *Oxytoxum* sp., *Gymnodinium* sp., *Ceratium* sp., and tintinnids were most abundant. Unfortunately, phytoplankton samples from the Saanich Inlet, at the time of collection of animals, were not taken. Therefore, it is not known whether the dominance of dinoflagellates in the food was because of their abundance in nature or was due to selective feeding. *Skeletonema costatum* was most abundant in the guts of animals fed in the laboratory. In any case, the second spawning indicates that the animals were getting adequate food in the laboratory.

Spawn:

The eggs of *Limacina helicina* were embedded in a thin, gelatinous ribbon, 3-4 mm long and 1-2 mm wide. The matrix was colorless and transparent but the eggs were slightly yellow, and were fairly closely spaced. The egg diameter was 95-100μ in the longest dimension, while the diameter of the ovum was 75μ. Because of the transparency and thinness of the ribbon, it was possible to estimate the egg number fairly accurately. Five complete egg ribbons and 4 egg-ribbon fragments were measured, their areas were estimated and the total number of eggs was counted. The mean egg-number per mm^2 was 80 and and the total egg-number per spawn was 500 to 700 (Table 1). The second spawn contained the same number of eggs.

Fol (1875) has described the spawns of several species of pteropods. The eggs are generally embedded in a gelatinous, transparent ribbon, or in a beaded string, or in a pea-pod-shaped case. The length of the egg ribbon varies from 2 to 10-50 mm (in *Cavolinia tridentata* (Forskål, 1773)), while 20 to 725 eggs are produced with each spawn. A small number of eggs per spawn is usually associated with large-sized eggs. *Limacina retroversa* produces a 2 mm-long egg ribbon containing approximately 300 eggs (calculated from figure 1 in Lebour, 1932). Most pteropods have a pelagic larval stage in the life history, but *L. helicoides* Jeffreys, 1877 is known to be a viviparous species (Bonnevie, 1913).

Table 1

Egg-Mass of *Limacina helicina* Phipps

Length (mm)	Width (mm)	Total Area (mm^2)	Eggs/mm^2	Total Number of Eggs
3.72	2.05	7.61	80.3	611
4.09	2.08	8.52	83.1	708
3.42	1.71	5.86	79.4	465
3.59	1.51	5.41	92.1	498
3.66	1.79	6.54	82.8	542
2.33[F]	1.86	4.32	76.1	
3.06[F]	1.24	3.80	88.8	
2.34[F]	2.05	4.80	73.4	
2.46[F]	1.69	4.15	78.0	
		Averages:	81.5	564.8

[F] Fragments of the egg-mass.

Microscopic examination of the crushed gonadial tissues of *Limacina helicina* showed ova of various diameters. This observation and the second spawning, 20 days after the first, suggest that this species apparently does not spawn out after a short period of intensive reproductive activity. Hsaio (1939) observed that *L. retroversa* lays few eggs at a time and the female reproductive cells of various ages are found in the ovotestis of the animal. Fol (1875) also noted such a protracted spawning in other species of pteropods.

Development:

No effort was made to study the details of the embryogenesis. Fol's (1875) information on the embryogenesis and organogenesis of pteropods has been summarized recently by Raven (1958).

The embryos began developing immediately (Figure 1 a, 1 b, 1 c). Toward the end of the first day, they started actively rotating inside the egg membrane. Unlike *Lima-*

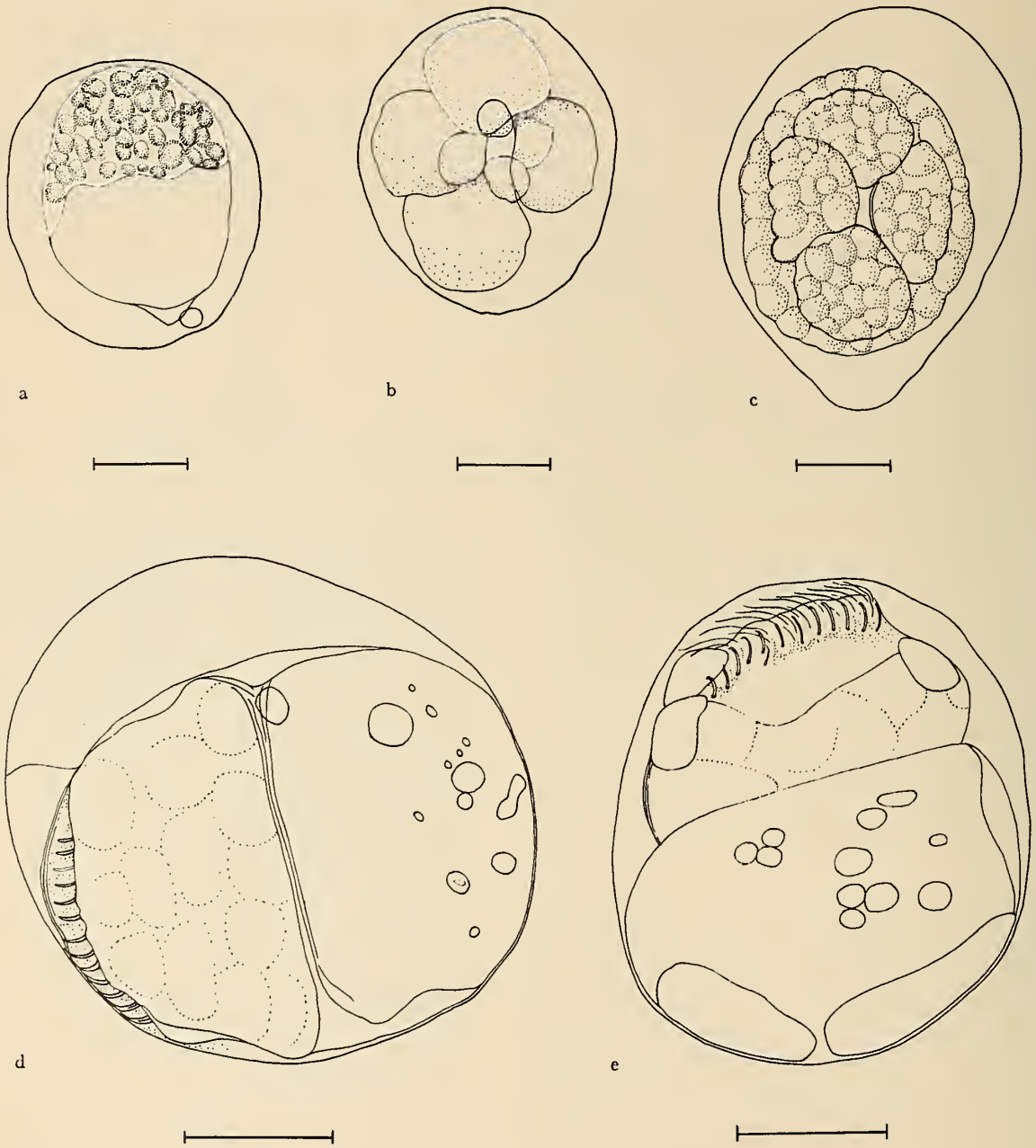

Figure 1

a, b, c: Stages in the development of the egg.
d, e: Egg, just before hatching.
Scale bar is 25 μ

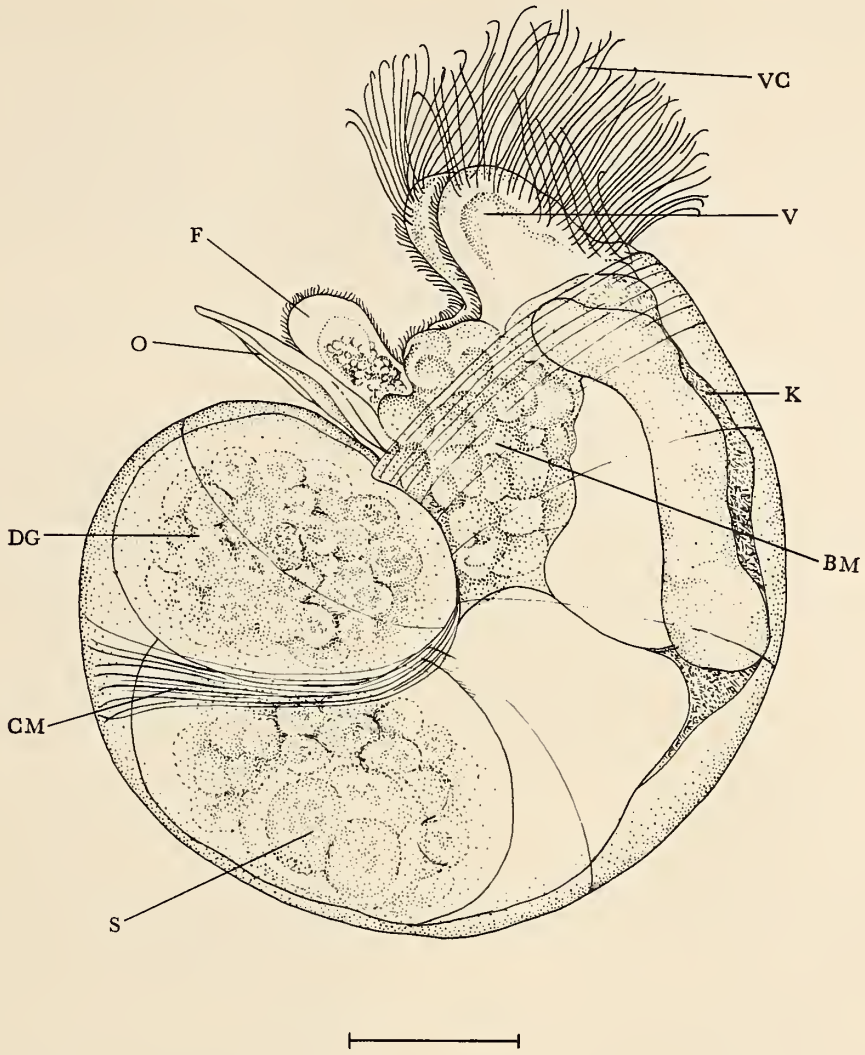

Figure 2

Seven-day-old veliger larva.
Scale bar is 25 μ
BM – Buccal Mass CM – Columellar Muscles F – Foot
K – Kidney O – Operculum S – Stomach V – Velum
VM – Velar Cilia

cina retroversa (Lebour, 1932), a cap-like, symmetrical and smooth shell developed before hatching (Figure 1 d, 1 e). Hatching occurred at the end of the second day. The newly hatched veliger larvae were about 75μ in diameter, with a bilobed velum, a small tongue-shaped, ciliated foot, and a thin, long, transparent operculum. The larvae were very active and soon after hatching congregated near the water surface or on the lighted side of the container. The apical mass contained all the internal organs. By the fourth day their shell diameter increased to about 105μ and the internal organs were clearly visible. By the seventh or eighth day, the shell

became slightly purple, and faint horizontal striations could be recognized under proper illumination. Besides an increase in size, no basic changes in the internal organs were apparent. Figure 2 shows the darkly pigmented kidney and some parts of the digestive system that were recognizable. Larvae attained a mean diameter of 200μ by the 30th day. The foot then had enlarged, and cilia of the velum had become stronger and longer. The operculum was still present, and larvae were still positively phototactic. When the veliger of *L. retroversa* becomes 0.32 mm in diameter, from the sides of the foot, two lappets develop, which grow to form the wings of the adult. As these lappets grow in size, the velum and the foot diminish (Lebour, 1932). No such signs of metamorphosis were seen even after 30 days in *L. helicina*. For lack of time, the experiment was terminated at the end of 30 days.

There was no difference in the growth of larvae placed in unfiltered sea water dominated by *Skeletonema costatum*, and of those grown in the filtered sea water supplemented with *Platymonas* sp. Larvae at all stages fed well and the food cells could be easily seen in the alimentary canal. An effort was made to induce metamorphosis by subjecting the larvae to a sudden temperature change. On the 25th day, the temperature of one batch of larvae was raised to 18°C for 12 hours and that of another was dropped to 4°C for the same length of time. All the larvae survived the temperature change but the process of metamorphosis was not initiated. Prior to or after the temperature change, no larval shell showed any signs of vertical striations, which are characteristic of *L. helicina* Variety A (McGowan, 1963).

SUMMARY

The euthecosomatous pteropod *Limacina helicina* produces a 3 - 4 mm long egg ribbon containing 500 - 700 eggs. The adults spawn more than once and apparently their reproductive activity is not limited to a short period. Development of veliger larvae up to 30 days is described and some observations on the food of the adult animals are recorded.

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